A Review on High Performance Liquid Chromatography (HPLC)

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ABSTRACT

Chromatography is defined as a set of techniques which is used for the separation of constituents in a mixture. This technique involves 2 phases one is stationary phase and another is mobile phase. The separation of constituents is based on the difference between partition coefficients of the two phases. The chromatography term is derived from the Greek words namely **chroma** which means "***color***" and **graphein** means***" to write***". High performance liquid chromatography (HPLC) is an essential analytical tool in assessing drug product. HPLC methods should be able to separate, detect, and quantify the various drugs and drug related degradants that can form on storage or manufacturing, detect and quantify any drugs and drug-related impurities that may be introduced during synthesis. Validation is the process of establishing the performance characteristics and limitations of a method and identification of the influences which may change these characteristics and to what extent. This article discusses the strategies and the issues pertinent to designing HPLC method development and validation.

INTRODUCTION

High Performance Liquid Chromatography which is also known as **High Pressure Liquid Chromatography**. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture. HPLC is an advanced technique of column liquid

chromatography. The solvent usually flows through column with the help of gravity but in HPLC technique the solvent will be forced under high pressures up to 400 atmospheres so that sample can be separated into different constituents with the help of difference in relative affinities [1-7] . In HPLC, pumps will be used to pass pressurized liquid solvent including the sample mixture which is allowed to enter into a column filled with solid adsorbent material. The interaction of each sample component will be varies and this causes difference in flow rates of each component and finally leads to separation of components of column. Chromatography can be depicted as a mass exchange process including adsorption. HPLC depends on pumps to pass a pressurized fluid and an example blend through a section loaded with adsorbent, prompting the partition of the specimen segments. The dynamic segment of the section, the adsorbent, is regularly a granular material made of solid particles (e.g. silica, polymers, etc.) 2 μm to 50 μm in size. The segments of the example mixture/blend are isolated from each other because of their distinctive degrees of connection with the retentive particles. The pressurized fluid is commonly a blend of solvents (e.g. water, acetonitrile and/or methanol) and is known as 'mobile phase'. Its organization and temperature plays an important part in the partition procedure by affecting the connections occurring between sample segments and adsorbent [8-15] . HPLC is recognized from traditional ("low weight") liquid chromatography because operational pressures are fundamentally higher (50 bar to 350 bar), while normal liquid chromatography regularly depends on the power of gravity to pass the portable stage through the segment. Because of the small sample amount isolated in scientific HPLC, column section measurements are 2.1 mm to 4.6 mm distance across, and 30 mm to 250 mm length. Additionally, HPLC segments are made with smaller sorbent particles (2 μm to 50 μm in normal molecule size). This gives HPLC high determining or resolving power (the capacity to recognize components) while isolating mixtures, which makes it a prominent chromatographic method.

PRINCIPLE

- HPLC (high performance liquid chromatography) is a chromatography technique where the mobile phase is a liquid and the stationary phase is packed into a stainless steel column at high pressure. It is usually silica particles mainly spherical nowadays. The efficiency is better when the particles are smaller typically 5um. A pump is used to push the solvent through the column and a detector with a flow-through cell used to measure

the separated peaks. Usually a computer with integration software collects the data and help quantifying the components.

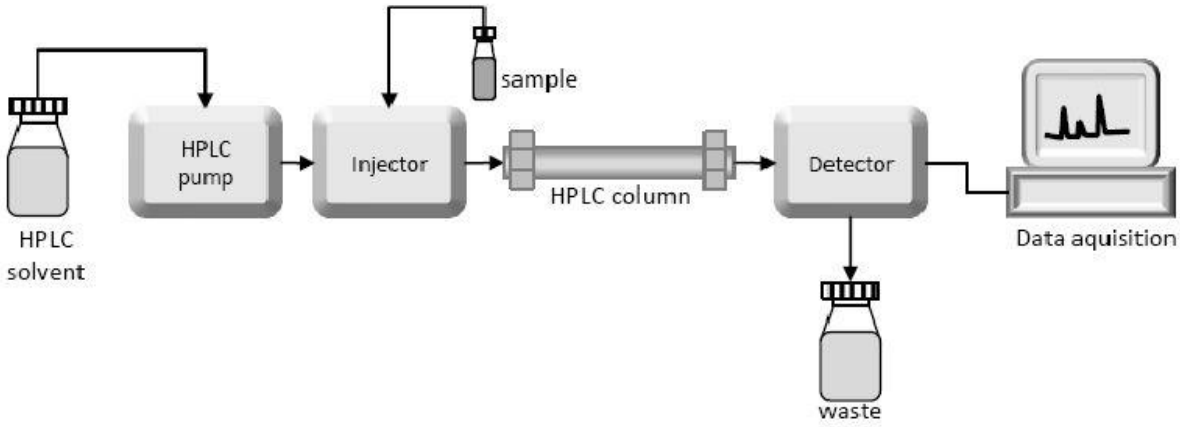
- HPLC works on the principle that some molecules take longer than others to pass through a chromatography column. This depends on the affinity of the molecule with the mobile phase (liquid or gas) and the stationary phase (solid or liquid). The ones that have more affinity with the stationary phase take longer to pass through and vice versa.
- HPLC is an analytical and as well a preparative technique where a liquid is pumped through a bed of very finely packed particles. The analytics in the mobile phase are interacting with the chemical groups on the particles. Some analytes will have a higher affinity than others for the stationary particles and thus will permit the separation of the various analytics. The beginning of HPLC goes back in the 60's where Jim Waters designed a refractive index detector for Dow Chemicals
- Reverse phase chromatography occurred in the 70's were chemist were able to install some hydrophobic chains initially C8 and then C18 (a chain of 18 Carbons long) on the silica particles. If the carbon load is sufficient, the properties are completely changed, the column becoming a non-polar one or hydrophobic. The mobile phase used is now a very polar one like water, methanol, acetonitrile Isopropanol.

WORKING

A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate

of mobile phase, typically milliliters per minute. An injector [sample manager or auto sample is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column. The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to *see* the separated compound bands as they elute from the HPLC column [most compounds have no color, so we cannot see them with our eyes]. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography [discussed in the section on HPLC Scale]. Note that high-pressure tubing and fittings are used to interconnect the pump, injector, column, and detector components to form the conduit for the mobile phase, sample, and separated compound bands. The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitate the concentration of the sample constituents (see Figure F). Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb ultraviolet light, a UV-absorbance detector is used. If the compound fluoresces, a fluorescence detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an evaporative-light-scattering detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a mass spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

INSTRUMENTATION



The HPLC instrumentation involves pump, injector, column, detector, integrator and display system. In the column the separation occurs. The parts include:

- **Solvent Reservoir**: The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture of polar and non-polar liquid components. Depending on the composition of sample, the polar and non-polar solvents will be varied.

- **Pump**: The pump suctions the mobile phase from solvent reservoir and forces it to column and then passes to detector. 42000 KPa is the operating pressure of the pump. This operating pressure depends on column dimensions, particle size, flow rate and composition of mobile phase.

- **Sample Injector**: The injector can be a solitary infusion or a computerized infusion framework. An injector for a HPLC framework should give infusion of the fluid specimen inside the scope of 0.1 ml to 100 ml of volume with high reproducibility and under high pressure (up to 4000 psi).

- **Columns**: Columns are typically made of cleaned stainless steel, are somewhere around 50 mm and 300 mm long and have an inward distance across of somewhere around 2 and 5 mm. They are generally loaded with a stationary phase with a molecule size of 3 µm to 10 µm. Columns with inner diameters of <2 mm are regularly alluded to as microbore segments.

Preferably the temperature of the mobile phase and the column should be kept consistent during investigation.

• **Detector**: The HPLC detector, situated toward the end of the column distinguishes the analysis as they elute from the chromatographic column. Regularly utilized detectors are UV-spectroscopy, fluorescence, mass spectrometric and electrochemical identifiers.

• **Data Collection Devices or Integrator:** Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic information. The PC coordinates the reaction of the indicator to every part and places it into a chromatograph that is anything but difficult to interpret. The schematic representation of a HPLC instrument ordinarily incorporates a sampler, pumps, and a locator. The sampler brings the sample into the mobile phase stream which conveys it into the column. The pumps convey the mobile phase through the column. The detector generates a sign relative to the measure of sample component rising up out of the segment, consequently taking into consideration quantitative investigation of the example parts. A computerized microchip and software control the HPLC instrument and give information data. A few models of mechanical pumps in a HPLC instrument can combine numerous solvents in proportions changing in time, producing a synthesis slope in the portable stage. Most HPLC instruments likewise have a column broiler that considers altering the temperature at which the partition is performed.

Types of HPLC

Depending on the substrate used i.e. stationary phase used, the HPLC is divided into following types:

• **Normal Phase HPLC**- In this method the separation is based on polarity. The stationary phase is polar, mostly silica is used and the non-polar phase used is hexane, chloroform and diethyl ether. The polar samples are retained on column.

• **Reverse Phase HPLC**- It is reverse to normal phase HPLC. The mobile phase is polar and the stationary phase is non polar or hydrophobic. The more is the non-polar nature the more it will be retained.

• **Size-exclusion HPLC**- The column will be incorporating with precisely controlled substrate molecules. Based on the difference in molecular sizes the separation of constituents will occur.

• **Ion-exchange HPLC**- The stationary phase is having ionically charged surface opposite to the sample charge. The mobile phase used is aqueous buffer which will control pH and ionic strength.

Applications of HPLC

The HPLC has several applications in the fields of pharmacy, forensic, environment and clinical. It also helps in the separation and purification of compound.

• **Pharmaceutical Applications:**

The pharmaceutical applications include controlling of drug stability, dissolution studies and quality control.

• **Environmental Applications:** Monitoring of pollutants and detecting components of drinking water.

• **Forensic Applications:** Analysis of textile dyes, quantification of drugs and steroids in biological samples.

• **Food and Flavor Applications:** Sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, analysis of preservatives.

• **Clinical Applications:** Detecting endogenous neuropeptides, analysis of biological samples like blood and urine.

CONCLUSIONS

It can be concluded from entire review that HPLC is a versatile , reproducible chromatographic technique used for the estimation of drug products. It has a wide applications in different fields in term of qualitative and quantitative estimation of active molecules. The HPLC is mostly used analytical technique. It is having several advantages. With the use of HPLC one can produce extremely pure compounds. It can be used in both laboratory and clinical science. With the use of HPLC the accuracy, precision and specificity can be increased. The only disadvantage of HPLC is high cost.

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